Document No : IRG/WP/1319

TEE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Working Group Ia Biological Problems (Flora)

Biochemical Aspects of White-Rot and Brown-Rot Decay

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Prepared for the Eighteenth Meeting
Honey Harbour, Ontario, Canada

May 17-22, 1987

IRG Secretariat
Drottning Kristinas väg 47c
S-114 28 Stockholm
Sweden

18 March 1987

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SUMMARY

This paper presents an overview of the decomposition of wood by white- and brown-rot fungi—the most important and potent of known wood-decay fungi. These organisms are unique among cellulose destroyers because of their strong capability to enzymatically degrade lignified material. Special emphasis is given to the following aspects of wood decomposition by white- and brown-rot fungi: (1) effects on the chemical and physical properties of wood, (2) method of invasion and ultrastructural modification of wood, (3) nature and activities of extracellular degrading enzymes, (4) relationship of ultrastructural changes to the degradative enzyme systems, and (5) unique physiological features of the fungi that can be used to control decay.

Key words: White-rot, brown-rot, wood-decay, decay fungi, cellulase, hemicellulase, ligninase, hyphal sheath, decay mechanisms

INTRODUCTION

Many different types of organisms deteriorate wood, but the greatest damage is microbiological--fungi and to a relatively minor extent, bacteria. Decay is by far the most serious kind of microbiological damage because it can cause structural failure--at times very rapidly. It is virtually impossible to accurately assess the monetary loss caused by decay that destroys wood products or impairs their aesthetic qualities; records are rarely kept. Treatments are available that can prevent or retard the destructive action of decay. Nevertheless, it is estimated that annual losses of over $1 billion in the United States result from fungal deterioration of untreated or inadequately treated wood (Scheffer, 1973).

Broad-spectrum metabolic poisons such as creosote, pentachlorophenol and arsenicals are currently used to protect wood from decay and, in general, are effective when properly applied. However, since the cost-effectiveness of these poisons has diminished and since they pose a threat to the environment, there is an urgent need for entirely different approaches to preventing wood deterioration. The unsophisticated approach of using metabolic poisons can only be phased out if new and more selective approaches can be developed. To do so rationally requires that we identify and take advantage of unique features of the degrading organisms or their biochemical systems. Identification of the biochemical agents in decay fungi responsible for degradation--either at the site of synthesis or

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secretion, or at the point of attack within the wood cell wall matrix--can lead to new, sharply targeted and environmentally benign wood preservation methods for controlling decay.

This paper presents an overview of the decomposition of wood by white- and brown-rot fungi--the most important and potent of the wood-degrading organisms. Special emphasis is given to their chemistry, biochemistry, and unique physiological features that can be manipulated to control decay.

NUTRITION AND COLONIZATION OF WOOD

The optimal nutritional needs of white- and brown-rot fungi vary, but the basic nutritional requirements are satisfied by the structural carbohydrates and certain extraneous materials in wood. Degradation of lignin does not occur in the absence of wood carbohydrates, and lignin is probably of limited importance as a carbon source (Kirk and Fenn, 1982). Successful colonization of wood depends largely on the ability of fungi to spread rapidly by utilizing nonstructural carbohydrates; the hyphae of both white- and brown-rot fungi are widespread in wood before any significant weight losses are detectable (Hulme and Shields, 1970).

Hyphae penetrate from cell to cell through natural openings in the wood (pits) or by boring holes in the cell wall. Early decay by brown- and white-rot fungi is characterized by the presence of at least one hyphal strand in each cell, suggesting that extracellular enzymes may not be able to diffuse outside the wood cells in which they are secreted (Wilcox, 1970). Thus, any physiochemical feature of the wood or its constituents that limits the ability of an organism to develop in the individual cells of wood will profoundly influence the wood's resistance to decay.

Nitrogen plays the most important role of the nonstructural nutrients in wood, and its part in wood decay has been extensively studied by Cowling and coworkers (Levi et al., 1968; Merrill and Cowling, 1966). The sparsity of nitrogen in wood (about 0.03-0.10% (Merrill and Cowling, 1966)) indicates that wood-decay fungi have an efficient mechanism for nitrogen metabolism and reuse (Levi et al., 1968). Field evidence (Larsen et al., 1978) and results of in vitro culture studies support the theory that wood-destroying Basidiomycetes can conserve and function with the small amount of nitrogen available in wood by autolysis and reuse of the nitrogen from their own mycelia or by the lysis of other fungi in wood during decay, together with an extremely economical use of nitrogen in metabolism. However, other sources of nitrogen, such as bacterial fixation, may sometimes be required to form sporophores. Studies of growth and nutrition of 42 white- and brown-rot fungi in synthetic media revealed that none of the fungi required nitrogen in organic form, but growth was usually greater with organic nitrogen than with ammonium salts (Jennison, 1952). Nitrate did not support the growth of 41 of these fungi.

Thiamine, the only vitamin essential for growth of most wood-decay fungi, is present in wood (Jennison, 1952). The discovery that most decay fungi require thiamine created interest in the possibility of protecting wood for above-ground service by alkaline treatments that

presumably destroy thiamine (Baechler, 1959). In laboratory tests designed to simulate above-ground exposure (low decay) alkaline-treated wood was resistant to brown-rot fungi but not white-rot (Highley, 1970). Additional study showed that the increased resistance of alkaline-treated wood to brown rot was caused by not only thiamine destruction but also the increased pH of the wood (Highley, 1973b).

EFFECT ON CHEMICAL AND PHYSICAL PROPERTIES OF WOOD

Various components of the wood cell wall are attacked differently by white- and brown-rot fungi. White-rot fungi utilize cellulose and the hemicelluloses at approximately the same rate relative to the original amounts present, whereas lignin is usually utilized at a somewhat faster rate on a relative basis. Recent evidence suggests that some white-rot fungi better utilize syringyl lignin residues than guaiacyl residues (Faix et al., 1985; Highley, 1982a; Leightley, 1984). Hardwood lignin contains both guaiacyl and syringyl lignin residues, but softwoods contain only guaiacyl residues (Freudenberg, 1968). Highley (1982a) found that Coriolus versicolor caused similar decay in a hardwood containing coniferous (guaiacyl) lignin and in softwoods. Faix et al. (1985) found that the white-rot fungus Phanerochaete chrysosporium preferentially degraded syringyl lignin residues. Using ^{13}C CP/MAS NMR analysis of decayed softwoods, Leightley (1984) found that white-rot and soft-rot fungi did not readily degrade the guaiacyl-type lignin, which may account for their slower attack in softwoods.

Brown-rot fungi utilize the hemicelluloses and cellulose of the cell wall, leaving the lignin essentially undigested. However, the fungi do modify lignin, as indicated by demethylation and the accumulation of oxidized polymeric lignin-degradation products. Chemical analysis of brown-rotted white pine (Pinus monticola) and hard maple (Acer rubrum) showed that the hemicellulose glucomannan is removed considerably faster than cellulose or xylan (Highley, 1987). Xylan is usually depleted faster than cellulose. Since hemicelluloses form an encrusting envelope around the cellulose microfibrils, which protects them against cellulolytic attack, further degradation and removal of depolymerized cellulose may depend on prior removal of the hemicelluloses. Thus, hemicellulose utilization may be a critical initial step in establishment of brown-rot fungi in wood. Consequently, prevention of hemicellulose utilization should inhibit the decay process.

White-rot fungi successively depolymerize cell wall substances only to the extent that the products are used for metabolism. They produce a gradual decrease in average degree of polymerization (DP) of holocellulose during all stages of decay and only slightly change the solubility properties of wood (Cowling, 1961). Brown-rot fungi, in contrast to white-rot, rapidly depolymerize holocellulose, and the degradation products are produced faster than they are utilized (Cowling, 1961). The rapid depolymerization of the wood carbohydrates is reflected by the substantial increase in alkali solubility products.

Brown-rot fungi cause a more rapid drop in strength properties than white-rot fungi, which reflects cellulose depolymerization. Early decay of both brown- and white-rotted wood is evidenced only by a loss in toughness. Wood that has lost only 3% of its weight because of decay will frequently exhibit a 50% loss in strength measured as toughness (Kennedy, 1958; Richards and Chidester, 1940). Reduction in toughness caused by brown rot could be explained by the sharp reduction in DP of cellulose during decay, but the basis for toughness loss caused by white rot lies elsewhere because these fungi reduce cellulose DP gradually.

While decaying wood, brown-rot fungi create a low pH, primarily by producing oxalic acid (Cowling, 1961). White-rot fungi also produce oxalic acid but they metabolize it, and so the pH is not as low.

MECHANISM OF WHITE-ROT DEGRADATION

Hyphae growing inside the cell lumens degrade the layers of the secondary wall from the inside, progressing successively from the S_3 layer outwards (Leise, 1970; Wilcox, 1970). The hyphae are often encased by gelatinous sheaths, which enable them to attach to the cell wall. The sheaths extend some distance from the hyphae, and cell walls are degraded beneath the sheaths. Although complete cell wall degradation generally occurs at the surface of the cell wall, TEM studies indicate that polymer-degrading enzymes diffuse from the cell wall of white-rot fungi to attack both polysaccharides and lignin (Eriksson et -- al., 1980; Highley et al., 1984; Highley and Murmanis, 1987; Ruel et al., 1981). Ericksson et al. (1980) found that material most likely of a hemicellulosic nature was removed at a distance of 3–4 nm from fungal hyphae of Sporotrichum pulverulentum Novo, now Phanerochaete chrysosporium Burds. TEM studies by Ruel et al. (1981) and Highley and Murmanis (1987) showed that lignin is morphologically changed at a distance from hyphae. The enzymes destroy the lamellar structure of lignin and produce aggregates in the form of granulae.

Cellulose Degradation

The enzyme mechanisms involved in cellulose degradation by the white-rot fungus, P. chrysosporium, have been extensively researched by Ericksson (1978). Results indicate that one or more endo-1,4-ß-glucanases act randomly over the exposed surfaces of cellulose microfibrils. The exposed nonreducing termini are then hydrolyzed by an exo-1,4-ß-glucanase, producing cellobiose. Cellobiose may be cleaved by a ß-glucosidase, yielding glucose. Exoglucanase and certain endoglucanases thus act synergistically--perhaps as a loose complex. Generally, the glucanases are repressed by high concentrations of simple sugars (Eriksson and Goodell, 1974; Highley, 1976). The glucanases have molecular weights ranging up to about 75,000 (Cowling and Brown, 1969; Cowling, 1975), but the ß-glucosidases are much larger (Ahlgren and Eriksson, 1967).

Cellulose degradation by white-rot fungi also involves oxidizing enzymes. A hemoprotein, cellobiose oxidase, oxidizes cellobiose to cellobionolactone, with O_2 serving as electron acceptor. This enzyme is responsible for the much more rapid hydrolysis of cellulose in

O_2 than in N_2 Cellobiose is also oxidized to cellobionolactone by cellobiose-quinone oxidoreductase, with quinones serving as electron acceptors. Also implicated in the overall process of cellulose degradation is a glucose oxidase that oxidizes glucose to glucono-lactone (Westermark and Ericksson, 1974). These various oxidizing activities seem to regulate the levels of glucose and cellobiose, and ultimately coordinate the rates of cellulose hydrolysis and metabolism of end products.

Eriksson (1981) suggests an additional mechanism for cellulose degradation in white-rot fungi, wherein hydrolysis by cellulases is combined with an oxidative step. Oxidative enzymes may produce reactants such as superoxide anion ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$), or hydroxyl radical ($OH\cdot$) that are involved in the primary attack of crystalline cellulose. $^1O_2$ and $OH\cdot$ are particularly reactive radicals (Pryor, 1976). These reactants are small enough to penetrate the microstructure of cellulose and predispose cellulose to endo- and exoglucanases produced by white-rot fungi. White-rot fungi have been reported to produce radicals that could react with cellulose (Ericksson, 1981; Nakatsubo et al., 1982). Nakatsubo et al. (1982) report $^1O_2$ formation by P. chrysosporium, and Eriksson (1981) found that $O_2^{\cdot -}$ is produced extracellularly by this fungi as well as other wood-destroying fungi. The origin of radical-generating oxidases and their involvement in cellulose degradation by white-rot fungi warrant further study.

Hemicellulose Degradation

Degradation of hemicelluloses proceeds in a manner roughly analogous to that of cellulose, but the mechanism of attack has been studied in much less detail. The hemicellulose chains are attacked first by endo-enzymes ("mannanases," "xylanases") that produce progressively shorter chains, which are hydrolyzed to simple sugars by glycosidases ("mannosidases," "xylosidases," "glucosidases"). It is not known whether exo-enzymes are involved. The enzymes involved in the removal of side-chain substituents (arabinose, uronic acids, acetyls) apparently have not been studied (Kirk and Cowling, 1984).

As with cellulases, simple sugars repress the production of most hemicellulose-degrading enzymes by white-rot fungi. Cellulose apparently is the only carbon source necessary to induce the formation of hemicellulose-degrading enzymes by white-rot fungi. Eriksson and Goodell (1974) proposed that a single regulatory protein governs the induction of cellulase, mannanase, and xylanase in Bjerkandera adusta (=Polyporus adustus). They further indicated that this single regulatory protein could be used to simply and effectively adjust the rate of wood decay by controlling enzyme induction.

Ahlgren and Eriksson (1967) studied some of the properties of hemicellulose-degrading enzymes of Haematostereum (Stereum) sanguinolentum and Heterobasidion annosum (=Fomes annosus). Mannanase was found to have a relatively large molecular size

compared to xylanase and cellulase, but all the endoenzyme molecules were smaller than their corresponding ß-glycosidases. Isoelectric separation showed that most of the hemicellulose-degrading-enzymes, like the cellulases, are proteins of acidic character. The pH optima of the hemicellulose-degrading enzyme are also in the acid range (pH 4-5).

Lignin Degradation

Rapid progress in understanding how lignin is degraded has been made in recent years through studies of the white-rot fungus P. chrysosporium, and this work has been extensively reviewed (Crawford and Crawford, 1984; Harvey et al., 1986; Kirk, 1984). The present paper briefly summarizes some of these findings.

The ligninolytic system of P. chrysosporium is not induced by lignin but appears constitutively as cultures enter secondary metabolism, that is, when primary growth ceases because of depletion of some nutrient (Kirk et al., 1978). Secondary metabolism is triggered by nitrogen, carbon, or sulfur limitation but not phosphorus limitation (Jeffries et -- al., 1981). Apparently the regulation of secondary metabolism, including lignin degradation, is connected with glutamate metabolism (Kirk, 1981). Cultures can obviously cope well with nitrogen limitation, so that sustained lignin degradation occurs. Kirk and Fenn (1982) speculate that lignin degradation is a secondary metabolic event because of the very low nitrogen content of wood (Merrill and Cowling, 1966). Soon after a white-rot fungus invades wood, nitrogen becomes limiting and secondary metabolism, including lignin degradation, begins.

The biochemical system that degrades lignin is extracellular, oxidative, and relatively nonspecific (Kirk and Shimada, 1985). White-rot fungi demethylate methoxyl groups in phenolic- and nonphenolic-containing lignin structures (Chen et al., 1982; Kirk and Adler, 1970) as well as hydroxylate aromatic rings (Kirk et al., 1970). Utilization of lignin by white-rot fungi apparently depends on the capacity to bring about the extracellular depolymerization of lignin, via carbon-carbon bond ether cleavage reaction (Kirk, 1983), in addition to preventing repolymerization between fragmented units.

Many of the chemical changes that white-rot fungi produce in lignin can be produced by ligninases, extracellular haernprotein peroxidases produced by P. chrysosporium (Gold et al., 1984; Tien and Kirk, 1984). The catalytic cycle of ligninases seems similar to that of other peroxidases (requires H_2O_2 but not O_2), but the ligninases are more powerful oxidants than previously studied peroxidases. The initial reaction in the attack on lignin models is a one-electron oxidation that generates relatively stable aromatic radical cations in the lignin polymer (Harvey et al., 1986; Kersten et al., 1984). Once generated by ligninase, the radical cations act as one-electron oxidants which can oxidize sites remote from the enzyme.

Harvey et -- al. (1986) propose that veratryl alcohol acts as a mediator of single-electron oxidation with ligninase. Ligninase oxidizes veratryl alcohol to a radical cation which could mediate the oxidation of lignin at a distance from the enzyme. These authors further

propose that these effects would be significantly accelerated in the presence of oxygen if perhydroxyl radicals, which can also act as single-electron oxidants, are produced. Of critical importance to this model of lignin breakdown is low acidity. Harvey et al. speculate that prior formation of sugar acids from cellulose breakdown is important in maintaining low acidity.

Evidence from several laboratories (Ander and Ericksson, 1985; Faison and Kirk, 1983; Forney et al., 1982a,b; Reddy et al., 1983) supports an essential role for H_2O_2 in lignin degradation by P. chrysosporium. Hydrogen peroxide is formed simultaneously with the ligninolytic systems, and when destroyed by catalase, the lignin-degrading capacity is also reduced (Faison and Kirk, 1983). Glucose oxidase is apparently the predominant source of H_2O_2 production in P. chrysosporium (Ericksson et al., 1986; Kelly et al., 1986). Glucose-oxidase activity in this fungus appears to be a secondary metabolic event and is triggered in response to nitrogen or carbohydrate starvation (Eriksson et al. 1986; Kelley et al., 1986).

Most of the detailed mechanistic studies on ligninases have been conducted using simple model compounds, and mechanisms by which ligninases could react with the complex structure remain unclear. In fact, neither the purified ligninases nor the crude filtrate duplicates the extensive extracellular degradation of lignin observed in intact ligninolytic cultures. Thus, components of the isolated lignin-degrading biochemical machinery are missing and need to be identified. The Institute for Microbial and Biochemical Technology at the U.S. Forest Products Laboratory (FPL) is actively searching for these missing ingredients.

MECHANISM OF BROWN-ROT DEGRADATION

Attack of the cell wall by brown-rot fungi, similar to that by white-rot, is predominantly initiated by hyphae growing in the lumina in contact with the tertiary wall (S_3) (Liese, 1970; Wilcox, 1970). Hyphae of brown-rot fungi, like those of white-rot, are often attached to the wood cell wall by gelatinous sheaths. However, degradation of the cell wall differs from white-rot in that the S_2 layer is intensely degraded whereas the S_3 layer remains relatively undegraded. Intense degradation occasionally occurs in localized areas of the middle lamella and cell comers without noticeable degradation of the surrounding secondary wall (Highley et al., 1985; Messner and Stachelberger, 1984). Enzymatic attack is not localized near the hyphae. Apparently, there is widespread and deep diffusion of the degrading catalysts.

Cellulose Degradation

The brown-rot fungi degrade cellulose in an unusual manner that differs from that of other cellulolytic organisms. They cause a rapid and extensive depolymerization (to the "limit" or crystallite DP at low weight loss), and thus are very destructive (Cowling, 1961). Acid hydrolysis has a similar effect on cellulose, as do various strong oxidants. The cellulose that remains has an average

DP of 150-200 and is more crystalline than non-depolymerized material because the cleavages occur in the amorphous non-crystalline regions. The biochemical agent--or the system that produces it--causing this initial depolymerization is clearly a small diffusible agent because enzymes are too large to penetrate wood to reach cellulo-e (Cowling, 1961; Cowling and Brown, 1969; Highley et al., 1983). Furthermore, cellulase preparations from brown-rot fungi generally exhibit very little activity toward insoluble crystalline celluloses (Highley, 1973a). However, brown-rot fungi possess an enzyme (endoglucanase) capable of hydrolyzing soluble celluloses, such as carboxymethy-cellulose. Endoglucanase is evidently common to all cellulolytic microorganisms. Attempts to identify the cellulose depolymerizing agent produced by brown-rot fungi have frustrated researchers for years. Identification of this agent could lead to methods that inhibit it and thus prevent wood decay.

Cowling and Brown (1969) suggest that the depolymerization of cellulose under physiological conditions with H_2O_2 and ferrous salts ('Fenton's reagent'), described by Halliwell (1965) might also apply to the mechanism of cellulose degradation employed by brown-rot fungi. Subsequent studies by Koenigs (1972, 1974) showed that brown-rot fungi produce H_2O_2, and that wood contains enough iron for a possible involvement of an iron/peroxide system in cellulose degradation. The change in DP by H_2O_2/Fe^{2+} in pine and sweetgum woods was similar to that produced by acid hydrolysis or brown rot. The alkali solubility curve of wood treated with H_2O_2/Fe^{2+} nearly fit that of brown-rotted wood--increasing rapidly at first, then slowly--and differed from the curve for acid-hydrolyzed cellulose. Schmidt et al. (1981) reported that oxalic acid, which is secreted by brown-rot fungi (Takao, 1965), reduces the Fe^{+3}--the species normally present in wood--to Fe^{+2}, the active form in Fenton's reagent. Formation of the powerful oxidant OH· from H_2O_2 could then rapidly depolymerize cellulose. Cobb, Whitten and Nicholas (unpublished) later demonstrated degradation of ^{14}C-cellulose by Gloeophyllum trabeum through a membrane with a nominal molecular exclusion limit of 1000.

Highley (1977) partially characterized brown-rotted cellulose, finding it to be oxidized and depolymerized. The fungus-degraded cellulose and cellulose oxidized by Fenton's reagent had several similar characteristics. The DP was drastically reduced (>90%) and solubility in 1% NaOH was similar (30%). Significantly, both types of cellulose had high and similar copper numbers and carboxyl contents. The carboxyls were not in uronic acid residues in the brown-rotted samples, indicating that they were in cleaved pyranose rings, or at C-4 or C-1 of the chain terminal residues. The carboxyl content was such that both chain-ends of all chains contained carboxyl groups. Cellulose oxidized by Fenton's reagent ($H_2O_2+Fe^{2+}$) was not examined for uronic residues in this study, but had been studied earlier by Ivanov et al. (1952), who found both uronic and non-uronic carboxyls. Together these studies suggest that the

biochemical agent of the brown-rot fungi is similar but not identical to Fenton's reagent.

Both chemical and microscopic analysis of brown-rotted wood and cellulose therefore point to involvement of a freely diffusible, small depolymerizing agent, at least in the initial stages of decomposition. Where does this agent come from? The possibility that H_2O_2 is the source has already been discussed. However, the cytochemical reagent diaminobenzidine did not detect H_2O_2 within or outside cells of the brown-rot fungus P. placenta during degradation of hemlock (Highley and Murmanis, 1986). Also, several brown-rot fungi did not produce extracellular H_2O_2 on various media (Highley and Murmanis, 1985; Highley, 1982b); H_2O_2 production was later induced by carbohydrate and nitrogen starvation in most of the brown-rot fungi (Highley, unpublished). Thus, with proper culture conditions (i.e. conditions under which cellulose is depolymerized), the brown-rot fungi may be induced to form H_2O_2. Oxygen may also be a factor in H_2O_2 induction. For example, Faison and Kirk (1983) found that H_2O_2 production by the white-rot fungus P. chrysosporium was barely detectable when the fungus was grown under atmospheric oxygen but substantially increased with increased oxygen. Another possibility is that other types of radicals, such as the superoxide radical $O_2\cdot$ or strongly oxidizing species resembling OH·. Thompson and Corbett (1985) treated cotton cellulose with potassium superoxide in dimethylsulfoxide and found that the bonding between fibrillar components was weakened, and DP was decreased from 6,700 to about 300. The superoxide radical itself is a rather weak oxidant, and these researchers proposed that the superoxide forms an intermediate but stronger radical that initiates a chain reaction that propagates through the amorphous regions of the fibers. Although the mechanism may differ, the effects on cellulose are similar to those produced by brown-rot fungi (Koenigs, 1974).

By exploring the oxygen biochemistry of brown-rot decay, scientists at FPL hope to identify the mechanism employed by these fungi that is responsible for the initial, rapid depolymerization of cellulose.

Hemicellulose Degradation

Brown-rot fungi secrete a number of hemicellulose-degrading enzymes, and the mechanism of cellulose breakdown appears similar to that of white-rot fungi (Highley, 1976; Keilich et al., 1970; King, 1966). However, little definitive work has been done on the hemicellulases of brown-rot fungi with respect to specificity, molecular size, and other properties such as active sites. The few hemicellulose-degrading enzymes that have been isolated and "purified" from brown-rot fungi are not substrate specific. A purified ß-glucosidase from the brown-rot fungus Gloeophyllum trabeum also hydrolyzed ß-xyloside (Herr et-- al., 1978); the purified xylanase from the brown-rot fungus Tyromyces palustris also had endoglucanase activity bur no glyco-sidase activity (Istihara et -- al., 1978). The purified endoglucanase from the brown-rot fungus Polyporus schwenitzii was accompanied by mannanase and xylanase activities (Keilich et al., 1970). A multiple

glycan and glycoside hydrolase was isolated from the brown-rot fungus, Poria placenta (Highley et al., 1981). However, glycanase activities were later separated from glycosidase activities (Green et al., 1987).

Like hemicellulases from white-rot fungi, the brown-rot enzymes have acidic pH optima and similar molecular weights (3-6 x 10^4). Thus, these enzymes would be too large to diffuse into the transient capillaries of wood. The hemicellulase complex isolated from Poria placenta was quite stable to temperature and pH effects, metals, chelators, and sulfhydryl compounds (Highley and Wolter, 1982). The xylanase fraction of this complex was resistant to denaturation or inactivation by SDS-2ME, urea, and guanidine-HCl (Green et al., 1987). The carbohydrate content of the xylanase complex ranged between 32% and 59% (w/w), and the stability is likely caused by the protective capacity of the high carbohydrate content.

Brown- and white-rot fungi differ in their regulation of hemicellulase synthesis. Many brown-rot fungi exhibit good hemicellulase as well as cellulase activities during growth on simple sugars, whereas white-rot fungi do not (Eriksson and Goodell, 1974; Highley, 1976). Regulation in wood-decay fungi, however, has not been studied in great detail and warrants additional study to obtain clues for controlling decay fungi.

Lignin Degradation

Chemical analysis of brown-rotted wood indicates that brown-rot fungi do not utilize lignin to an appreciable extent (Cowling, 1961; Kirk and Highley, 1973). Their main effect on lignin is demethylation of aryl methoxyl groups (Kirk and Adler, 1970), although oxidative changes occur, including some cleavage of aromatic rings (Kirk, 1975). Cowling (1961) also showed that lignin decayed by P. placenta has appreciably greater solubility in water and 1% NaOH than lignin in sound wood. Haider and Trojanowski (1980) demonstrated that brown-rot fungi can be induced to metabolize lignin somewhat. They found that isolated lignins are degraded to CO_2 to a limited extent by brown-rot fungi in liquid culture. Microscopic studies (Highley et al., 1985) show that brown-rot fungi cause degradation of the cell wall, including the lignin-rich middle lamella and cell corners. Thus, brown-rot fungi may have a greater ligninolytic capacity than thought from earlier studies. The ligninolytic agent produced by brown-rot fungi is possibly the same or similar to the degrading agent that initiates the rapid depolymerization of cellulose.

Harvey et al. (1986) discuss the possibility that the brown-rot fungi degrade lignin by a single-electron oxidation similar to that of white-rot fungi. They both demethylate methoxyl groups in phenolic- and nonphenolic-containing lignin structures (Kirk and Adler, 1970; Kirk and Chang, 1974) as well as hydroxylate aromatic rings (Kirk et al., 1970). Both types of decay fungi cause Cu-side chain oxidations and aromatic ring cleavage reactions (Kirk, 1975; Kirk and Adler, 1970; Kirk and Chang, 1975). They differ, however, in that the oxidative processes caused by brown-rot fungi result in the formation of polymeric fractions of lignin that have higher

the formation of polymeric fractions of lignin that have higher molecular weight than those from sound lignin (Kirk, 1975). Harvey et al. (1986) propose that brown-rot fungi repolymerize lignin because highly unstable radical cation intermediates formed from phenolic-containing aromatic compounds are oxidized by a single electron. These phenoxy radicals undergo further differing reactions, including oxidative carbon-to-carbon and carbon-to-oxygen coupling reactions, to produce higher molecular weight products that the brown-rot fungi evidently cannot metabolize.

ROLE OF HYPHAL SHEATHS

Extracellular layers (hyphal sheaths) enveloping hyphae of wood decay fungi are a common morphological feature (Palmer et al., 1983a,b). Hyphal sheaths are found around the growing points and along the length of hyphae, and encircle conidia and chlamydospores, but they are not present on necrotic hyphae (Palmer et al., 1983a,b). Structurally, the hyphal sheath in white-rot fungi does not differ from that of brown-rot fungi. Fundamental differences in the chemical nature of the hyphal sheath in white-rot fungi as opposed to brown-rot fungi are also unlikely. Mucilages and gels associated with vegetative hyphae of fungi in many taxa have been identified histochemically as polysaccharides (Bullock et al., 1980; Dowsett, 1981; Foster, 1981; Green et al., 1980; Moore-Landecker, 198l), and a water-soluble ß-1,3-ß,6-linked glucan surrounds hyphae of the white-rot fungus Schizophyllum commune (Van der Valk et al., 1977). Francis and Leightley (1983) used solid-state ^{13}C-NMR to examine the nature of the hyphal sheath around the brown-rot fungus Coniophoria puteana and the white-rot fungus Trametes lactinea. They found the extracellular layers around both types of fungi to be polysaccharide, consisting of glucan with base units of glucose. Thus, although formulae of sheaths may vary from fungus to fungus, the differences are not great.

Hyphal sheaths are certainly involved directly and indirectly in survival mechanisms of decay fungi. Sheaths are postulated to provide several functions for living vegetative hyphae (Palmer et al., 1983a,b; Sutter et al., 1983). First, sheaths may facilitate wood degradation by storing or concentrating degrading agents, and translocating the degrading agents for initial conditioning and subsequent attack on cell-wall polymers (Jutte and Sachs, 1976; Leightley and Eaton, 1980; Liese and Schmid, 1966; Proctor, 1941). The sheaths slow diffusion of the degrading agents and retard dispersion and loss. Concentration levels of degrading agents that will enable molecular or organismal interaction can be induced. The sheaths may also condition wood to degradation; e.g., by causing the cell wall to swell and thus predisposing it to enzymatic attack. For instance, an increase in the degree of swelling by wet fibrillation or by use of never-dried samples was found to reduce structural stress, and enzymatic hydrolysis was definitely enhanced because the fibrillar morphological units in cellulose were more accessible (Phillip et al., 1981). Results of a previous study were similar. King (1968) found that a cell-free enzyme preparation from the brown-rot fungus Coniophora cerebella weakly degraded dried holocellulose but readily degraded never-dried holocellulose. Thus, one or more interactions initiated or accomplished within sheaths may explain

why in situ attack of cell-wall polymers is so much more aggressive than that produced by cell-free enzyme preparations.

Secondly, the release of cellular contents from hyphae into sheaths may provide food supplies for growing hyphae. Sheaths are known to contain proteinaceous materials and polysaccharides (Bullock et al., 1980; Saito, 1974; Van der Valk et al., 1977), which can be used immediately or stored. Saito (1974) showed that the sheath (which he calls the outer fibrillar layer) rather than glycogen is utilized during gemination of sclerotia of Sclerotinia sclerotium. The sheath's proteinaceous compounds could supply nitrogen for wood-decaying fungi since wood has little nitrogen.

The third proposed function of sheaths is to attach the hyphae growing points, which secrete a gel, to solid substrates. As pointed out by Murray and Maxwell (1975), gels have long been known to affix appressoria to leaf surfaces. Hyphae of decay fungi can be removed from glass cover slips, but gel tracings remain on the glass (Palmer et al., 1983a,b). On substrates in aerial environments, hyphal contacts are more secure during periods of air movement. The sheaths extend laterally from hyphae, sometimes to considerable distances (Evans et al., 1981; Palmer et al., 1983a,b; Schmid and Liese, 1965); viscosity of the gels must be initially low and the depth minimal. Consequently, the contours of a substrate are often visible on the surface of the sheath gel. Thus, the sheath gel securely attaches hyphae to the substrate and permits a greater area for substrate-hyphae interaction.

The fourth postulated function of hyphal sheaths is to reduce hyphal dessication and nutrient loss. A viscous hyphal gel with a smooth surface retards vaporization of water more effectively than an uncoated fibrillar cell wall, which would tend to be like a wick. The viscous hyphal gel thus provides longer survival in aerial habitats. The extensive and intensive contact of hyphae and substrate provided by sheaths increases the supply of nutrients by preventing their escape from the substrate; for example. protoplasmic structures from autolyzing hyphae can concentrate. Consequently in nitrogen-deficient substrates, such as wood, decay fungi may reutilize nitrogen in proteins that were not lost immediately in water films or degraded by other organisms.

Finally, hyphal sheaths may protect living hyphal cells against environmental agents that produce mechanical or chemical injury. They may provide an elasticity to aerial hyphae and thereby reduce breakage of cell walls as well as provide protection against damage caused by small, motile animals such as mites, and moving particles such as grains of sand or dust. Sheaths provide a physical and chemical deterrent to competitors such as bacteria and other fungi. Sutter et-- al. (1983) provide evidence that sheaths play a role in the detoxification of copper and other metallic ions in fungal substrates. They found that Poria placenta formed microcrystalline tubes composed of extracellular material and laminated deposits of calcium or copper oxalate, depending on whether the fungus had been growing on copper sulphate-treated wood or untreated wood.

Further research on determining the chemical nature and function of the hyphal sheath may elucidate some of the mysteries of wood decay.

Research in the chemistry and biochemistry of the decay process has accelerated primarily because of a desire to exploit cellulases and lignin-degrading enzymes of wood-decay fungi €or bioconversion processes. Less research has been directed toward understanding and controlling decay in wood products. For example, the recent discovery of ligninase and the apparently essential role of H_2O_2 in lignin degradation by white-rot fungi resulted from studies aimed at increasing decomposition of wood. The information gained from this research is equally valuable to those interested in preserving wood, and should provide insight into how to prevent lignin degradation and thus wood decay by certain fungi. Therefore, it is important that researchers concerned with protecting wood from decay keep informed of mechanistic studies of the decay process.

The digestion of wood is a unique physiological feature of wood decay fungi that can be used to control decay. Treatments that modify wood into a nonsubstrate (Rowell, 1984) or chemicals that interfere with formation, release, or activity of the degrading agents are possibilities for controlling decay.

It is possible to change the basic chemistry and, therefore, the properties of cell-wall constituents through chemical modification (Rowell, 1984). Chemical modification of wood for biological reactions is based on the premise that the degrading agents directly contact the substrate and the substrate has a specific configuration (Rowell, 1984). If the substrate is modified, this highly specific reaction cannot occur. However, biochemical studies indicate that the attack on wood cell-wall polymers may be initiated not by hydrolytic enzymes but by oxidative reactions, and in the case of lignin degradation the degrading agents are nonspecific. Thus, if nonspecific degrading agents are involved in wood degradation, modifying the substrate to make it undigestible may not be so easily accomplished. If future biochemical studies conclusively identify oxidative reactions as a key ingredient in wood degradation, then wood could be protected from decay by modification with chemicals based on their anti-oxidant properties.

Extracellular degrading enzymes, although of questionable importance in the initial stages of decay, are certainly important later in the breakdown of polymeric carbohydrates to small units that can be metabolized. Biochemical studies indicate that the extracellular degrading enzymes, such as cellulases and hemicellulases, produced by decay fungi are extremely stable, probably because these enzymes have a high carbohydrate content. Because of their extreme stability, it is unlikely that simply inactivating these enzymes would inhibit decay. An alternative means of controlling wood decay is to regulate formation of the degradative enzymes or prevent the fungi from secreting an active form of the enzymes. Little work has been conducted on enzyme regulation in decay fungi, but formation of cellulases and hemicellulases by white-rot fungi appears to be catabolically repressed (simple sugars prevent enzyme formation); however, this is not the case with several brown-rot fungi. Therefore, white-rot decay might possibly be controlled by treating wood with nometabolizable sugar analogs that interfere with enzyme

formation. It is unknown whether degradative enzymes are actively transported across the cell membrane or whether they are released upon autolysis of the mycelium. If secretion is an active process, it may be subject to inhibition by certain antibiotics and metabolic poisons.

Despite the considerable progress that has been made in understanding lignin and carbohydrate digestion by decay fungi, we still do not know how the degrading agents gain access to the substrates. Inability to gain access to polymers in the wood cell wall is probably the reason why cell-free extracts from fungi cause such weak attack on wood compared to in situ attack by the organisms. There appears to be an intricate relationship between the degrading fungus and the substrate that permits aggressive degradation in situ that is lost during preparation of cell-free extracts. Is the missing ingredient the extracellular gelatinous sheath? Identification of this unknown factor might well provide us with the "Achilles heel" in the decay process that can be used to develop new decay inhibitors.

Information from physiological and biochemical studies can also be utilized for specific inhibition of fungal growth and development. One approach is the utilization of chemicals that prevent synthesis of chitin, which is an essential component of fungal cell walls (Johnson and Chen, 1983). Another attractive approach is to identify the micro-elements or growth factors in wood essential for fungal growth, such as thiamine, with a view rendering these growth factors unavailable. Although wood contains all the micro-elements essential for growth and development of wood decay fungi, certain unidentified growth substances in wood greatly stimulate fungal growth when added to a "complete" medium. These beneficial substances deserve a closer look because their destruction or alteration in wood could lead to improved control methods against decay. Lastly, studies at FPL are being conducted on the regulation of nitrogen metabolizing enzymes by specific enzyme inhibitors. Preliminary data (Illman, personal communication) indicates that "suicide" inhibitors against several different enzymes responsible for polyamine synthesis and nitrogen assimilation are effective in preventing the growth and development of the brown-rot fungus P. placenta in vitro. Such "suicide" inhibitors or their analogs may have the potential to be effective fungicides for wood preservation.

Wood protection in the future will depend less on the use of broad-spectrum metabolic poisons. Biochemical and physiological studies of wood decay are revealing new and exciting information on mechanisms of decay, which may allow us to interfere with essential biochemical processes and thus develop new, sharply targeted, nonhazardous preservatives.

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